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Short communication

Seroprevalence of *Neospora caninum* and *Toxoplasma gondii* in black-tailed deer (*Odocoileus hemionus columbianus*) and mule deer (*Odocoileus hemionus hemionus*)

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Abstract

Deer are considered important intermediate hosts for the coccidian parasites, *Toxoplasma gondii* and *Neospora caninum*. Antibodies to *N. caninum* and *T. gondii* were determined in sera of 42 mule deer (*Odocoileus hemionus hemionus*) and 43 black-tailed deer (*Odocoileus hemionus columbianus*) from Washington state, USA, using direct agglutination test with specific antigens. A titer of 1:25 was considered diagnostic for both parasites. *N. caninum* antibodies were found in 7 of 42 mule deer and 8 of 43 black-tailed deer. *T. gondii* antibodies were found in 14 black-tailed deer but not in any of the mule deer. This is probably the first report of seroprevalence of *N. caninum* in these hosts.
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1. Introduction

Neospora caninum and *Toxoplasma gondii* are related coccidians that can cause infection in many species of domestic and wild animals (Dubey and Beattie, 1988; Dubey et al., 2007). The white-tailed deer (*Odocoileus virginianus*) is considered an important intermediate host for *N. caninum* in the USA because the seroprevalence is very high (Dubey et al., 1999; Lindsay et al., 2002; Gondim et al., 2004; Anderson et al., 2007) and viable *N. caninum* has been isolated from this host (Vianna et al., 2005). As yet, there is no report of clinical neosporosis in the white-tailed deer but fatal neosporosis was reported in a black-tailed deer (*Odocoileus hemionus columbianus*) from California (Woods et al., 1994), in an Eld's deer (*Cervus eldi siamensis*) from France (Dubey et al., 1996), and a Fallow deer (*Dama dama*) from Switzerland (Soldati et al., 2004). In the present paper, we report seroprevalence of *N. caninum* in black-tailed deer and mule deer (*Odocoileus hemionus hemionus*), the first time from these hosts.

There are several reports of seroprevalence of *T. gondii* infection in white-tailed deer in the USA (Brillhart et al., 1994; Vanek et al., 1996; Lindsay et al.,

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1991; Humphreys et al., 1995; Dubey et al., 2004), and viable *T. gondii* has been isolated from hunted deer (Lindsay et al., 1991; Dubey et al., 2004, 2008). Cases of clinical toxoplasmosis (Sacks et al., 1983), including ocular manifestations (Ross et al., 2001), have been documented in humans who had consumed undercooked venison. Antibodies to *T. gondii* were reported in 35% of 89 mule deer from Nebraska (Lindsay et al., 2005) and viable *T. gondii* was isolated from this host in Montana (Dubey, 1982). In the present paper, we report seroprevalence of *T. gondii* in black-tailed deer and mule deer. While mule and black-tailed deer share many characteristics and will readily interbreed if they have the opportunity, mule deer are found in xeric and open country while black-tailed deer are generally found in wetter, more heavily forested areas. In the state of Washington, mule deer and black-tailed deer are separated geographically by the Cascade Mountain range (Wallmo, 1981).

2. Materials and methods

2.1. Naturally exposed deer

Sera were collected from 7 mule deer that were killed by hunters, and from 35 mule deer and 43 black-tailed deer that were live-captured and handled for research purposes between 1998 and 2002. Sera were stored at −20 °C until examined for the present study in 2007. The five mule deer collection sites were: Coffee Pot Lake (47.49N, 118.56W) 7, Revere (47.07N, 117.92W) 13, Pacific Lake (47.41N, 118.71W) 5, Sanpoil (47.95N, 118.65W) 6, and Swawilla (47.92N, 118.74W) 13. The black-tailed deer were from McNeil Island (47.21N, 122.69W) 8, Vale Tree Farm (46.83N, 122.60W) 7, Indian Island (48.00N, 122.77W) 6, San Juan (48.35N, 123.10W) 9, Herron Island (47.27N, 122.83W)10, and site not recorded for 3 deer.

2.2. Neospora serology

Sera of deer were first tested for *N. caninum* antibodies using two-fold dilutions, from 1:25 to 1:3200 with the direct *Neospora* agglutination test (NAT) as described by Romand et al. (1998). Subsequently, sera were tested in 1:25 serum dilution by the indirect fluorescent antibody test (Dubey et al., 1988) using 1:25 dilution of the fluorescein-labelled affinity purified rabbit anti-white-tailed deer IgG (heavy and light chain) antibodies (KPL, Gaithersburg, MD). Appropriate controls were included using positive and negative sera from white-tailed deer.

2.3. Toxoplasma serology

Sera of deer were tested for *T. gondii* antibodies using two-fold dilutions, from 1:25 to 1:3200 with the modified agglutination test (MAT) as described by Dubey and Desmonts (1987). The MAT is not species specific.

3. Results and discussion

3.1. Mule deer

Antibodies to *N. caninum* were found in seven of 42 mule deer by NAT (Table 1). The three samples with high NAT titers (1:200, 1:400, 1:1600) were also positive by the IFAT. None of the 42 samples had *T. gondii* antibodies.

3.2. Black-tailed deer

Antibodies to *N. caninum* were found in 8 of 43 deer by the NAT; none were positive by the IFAT. *T. gondii*

Table 1
Antibodies to *N. caninum* and *T. gondii* in mule deer and black-tailed deer from USA

I.D.No.	Location	*N. caninum*		*T. gondii*
		NAT	IFAT	MAT
Mule deer				
2–17	Swawilla	25	Neg.	Neg.
2–22	Pacific Lake	50	Neg.	Neg.
2–23	Pacific Lake	50	Neg.	Neg.
2–27	Sanpoil	1600	Pos.	Neg.
2–30	Swawilla	400	Pos.	Neg.
2–31	Swawilla	25	Neg	Neg.
Black-tailed deer				
98–2	NR	Neg.	Neg.	200
98–24	McNeil Island	Neg.	Neg.	25
0–15	NR	Neg.	Neg.	1600
0–17	NR	25	Neg.	25
0–20	NR	Neg.	Neg.	3200
0–43	Vale Tree Farm	200	Not done	Neg.
ND-3	NR	Neg.	Neg.	1600
ND-3	San Juan	Neg.	Neg.	1600
ND-12	San Juan	Neg.	Neg	1600
ND-22	Herron Island	25	Neg.	50
ND-25	Herron Island	25	Neg.	800
ND-27	Herron Island	50	Neg.	800
ND-29	Herron Island	25	Neg.	800
ND-30	Herron Island	25	Neg.	800

NR is not recorded. ND is no date recorded. Neg. is <25.

antibodies were found in 14 of 43 deer. Seven of the seropositive deer had antibodies to both parasites (Table 1).

In the present study, antibodies to *N. caninum* and *T. gondii* were initially determined using respective direct agglutination tests. Although these tests have not been validated in the species of the deer tested in the present study, they appear to be parasite-specific in all hosts so far investigated. Of these, the *T. gondii* MAT has been extensively validated for pigs using the isolation of the parasite as the standard (Dubey et al., 1995; Dubey, 1997). In two studies, viable *T. gondii* was isolated from 61% and 67% of seropositive adult white-tailed deer (Lindsay et al., 1991; Dubey et al., 2004). Isolation of viable *N. caninum* is difficult from any host (Dubey et al., 2007). Therefore, none of the serological tests have been validated for the diagnosis of neosporosis using isolation of the parasite as the standard. In the present study, the three mule deer samples with relatively high NAT titers (1:200 or higher) were also positive with the IFAT; thus, *Neospora* antibodies were demonstrated with two serological tests. With respect to black-tailed deer NAT positive sera, the seven sera with low NAT titers were negative by IFAT; the serum with NAT titer of 1:200 was not tested by IFAT.

In the present study, all seven mule deer with *N. caninum* antibodies were seronegative for *T. gondii*, suggesting specificity of the tests. With black-tailed deer, of the 14 seropositive animals, seven were seropositive for only one parasite, also suggesting the specificity of the tests. Why the seroprevalence of *N. caninum* and *T. gondii* in the two species of the deer was different is unexplained and a larger sample size will be needed to verify the result.

References

Anderson, T., Dejardin, A., Howe, D.K., Dubey, J.P., Michalski, M.L., 2007. *Neospora caninum* antibodies detected in Midwestern white-tailed deer (*Odocoileus virginianus*) by Western blot and ELISA. Vet. Parasitol. 145, 152–155.

Brillhart, D.B., Fox, L.B., Dubey, J.P., Upton, S.J., 1994. Seroprevalence of *Toxoplasma gondii* in wild mammals in Kansas. J. Helminthol. Soc. Wash. 61, 117–121.

Dubey, J.P., 1982. Isolation of encysted *Toxoplasma gondii* from muscles of mule deer in Montana. J. Am. Vet. Med. Assoc. 181, 1535–11535.

Dubey, J.P., 1997. Validation of the specificity of the modified agglutination test for toxoplasmosis in pigs. Vet. Parasitol. 71, 307–310.

Dubey, J.P., Beattie, C.P., 1988. Taxoplasmosis of Animals and Man. CRC Press, Boca Raton, Florida, pp. 1–220.

Dubey, J.P., Desmonts, G., 1987. Serological responses of equids fed *Toxoplasma gondii* oocysts. Equine Vet. J. 19, 337–339.

Dubey, J.P., Hattel, A.L., Lindsay, D.S., Topper, M.J., 1988. Neonatal *Neospora caninum* infection in dogs: isolation of the causative agent and experimental transmission. J. Amer. Vet. Med. Assoc. 193, 1259–1263.

Dubey, J.P., Thulliez, P., Weigel, R.M., Andrews, C.D., Lind, P., Powell, E.C., 1995. Sensitivity and specificity of various serologic tests for detection of *Toxoplasma gondii* infection in naturally infected sows. Am. J. Vet. Res. 56, 1030–1036.

Dubey, J.P., Rigoulet, J., Lagourette, P., George, C., Longeart, L., LeNet, J.L., 1996. Fatal transplacental neosporosis in a deer (*Cervus eldi siamensis*). J. Parasitol. 82, 338–339.

Dubey, J.P., Hollis, K., Romand, S., Thulliez, P., Kwok, O.C.H., Hungerford, L., Anchor, C., Etter, D., 1999. High prevalence of antibodies to *Neospora caninum* in white-tailed deer (*Odocoileus virginianus*). Int. J. Parasitol. 29, 1709–1711.

Dubey, J.P., Graham, D.H., de Young, R.W., Dahl, E., Eberhard, M.L., Nace, E.K., Won, K., Bishop, H., Punkosdy, G., Sreekumar, C., Vianna, M.C.B., Shen, S.K., Kwok, O.C.H., Sumners, J.A., Demarais, S., Humphreys, J.G., Lehmann, T., 2004. Molecular and biologic characteristics of *Toxoplasma gondii* isolates from wildlife in the United States. J. Parasitol. 90, 67–71.

Dubey, J.P., Schares, G., Ortega-Mora, L.M., 2007. Epidemology and control of neosporosis and *Neospora caninum*. Clin. Microbiol. Rev. 20, 323–367.

Dubey, J.P., Velmurugan, G.V., Ulrich, V., Gill, J., Carstensen, M., Sundar, N., Kwok, O.C.H., Thulliez, P., Majumdar, D., Su, C., 2008. Transplacental toxoplasmosis in naturally-infected white-tailed deer: isolation and genetic characterization of *Toxoplasma gondii* from foetuses of different gestational ages. Int. J. Parasitol. 38, 1057–1063.

Gondim, L.F.P., McAllister, M.M., Mateus-Pinilla, N.E., Pitt, W.C., Mech, L.D., Nelson, M.E., 2004. Transmission of *Neospora caninum* between wild and domestic animals. J. Parasitol. 90, 1361–1365.

Humphreys, J.G., Stewart, R.L., Dubey, J.P., 1995. Prevalence of *Toxoplasma gondii* antibodies in sera of hunter-killed white tailed deer in Pennsylvania. Am. J. Vet. Res. 56, 172–173.

Lindsay, D.S., Blagburn, B.L., Dubey, J.P., Mason, W.H., 1991. Prevalence and isolation of *Toxoplasma gondii* from white-tailed deer in Alabama. J. Parasitol. 77, 62–64.

Lindsay, D.S., Little, S.E., Davidson, W.R., 2002. Prevalence of antibodies to *Neospora caninum* in white-tailed deer, *Odocoileus virginianus*, from the Southeastern United States. J. Parasitol. 88, 415–417.

Lindsay, D.S., McKown, R.D., DiCristina, J.A., Jordan, C.N., Mitchell, S.M., Oates, D.W., Sterner, M.C., 2005. Prevalence of agglutinating antibodies to *Toxoplasma gondii* in adult and fetal mule deer (*Odocoileus hemionus*) from Nebraska. J. Parasitol. 91, 1490–1491.

Romand, S., Thulliez, P., Dubey, J.P., 1998. Direct agglutination test for serologic diagnosis of *Neospora caninum* infection. Parasitol. Res. 84, 50–53.

Ross, R.D., Stec, L.A., Werner, J.C., Blumenkranz, M.S., Glazer, L., Williams, G.A., 2001. Presumed acquired ocular toxoplasmosis in deer hunters. J Ret. Vit. Dis. 21, 226–229.

Sacks, J.J., Delgado, D.G., Lobel, H.O., Parker, R.L., 1983. Toxoplasmosis infection associated with eating undercooked venison. Am. J. Epidemiol. 118, 832–838.

Soldati, S., Kiupel, M., Wise, A., Maes, R., Botteron, C., Robert, N., 2004. Meningoencephalomyelitis caused by *Neospora caninum* in a juvenile fallow deer (*Dama dama*). J. Vet. Med. A. 51, 280–283.

Vanek, J.A., Dubey, J.P., Thulliez, P., Riggs, M.R., Stromberg, B.E., 1996. Prevalence of *Toxoplasma gondii* antibodies in hunter-killed white-tailed deer (*Odocoileus virginianus*) in four regions of Minnesota. J. Parasitol. 82, 41–44.

Vianna, M.C.B., Sreekumar, C., Miska, K.B., Hill, D.E., Dubey, J.P., 2005. Isolation of *Neospora caninum* from naturally infected white-tailed deer (*Odocoileus virginianus*). Vet. Parasitol. 129, 253–257.

Wallmo, O.C., 1981. Mule and black-tailed deer distribution and habitats. In: Wallmo, O.C. (Ed.), Mule and Black-Tailed Deer of North America. University of Nebraska Press, Lincoln, Nebraska, USA, 605 pp., pp. 1–24.

Woods, L.W., Anderson, M.L., Swift, P.K., Sverlow, K.W., 1994. Systemic neosporosis in a California black-tailed deer (*Odocoileus hemionus columbianus*). J. Vet. Diagn. Invest. 6, 508–510.